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(54) Title: HAEMOPOIETIC MATURATION FACTOR (57) Abstract Disclosed is a human maturation factor polypeptide and DNA(RNA) encoding such haemopoietic maturation factor polypeptides. Also provided is a procedure for producing such polypeptide by recombinant techniques and antibodies against such polypeptide. Such polypeptides may be combined with a suitable pharmaceutical carrier or diluent to provide diagnostic, therapeutic and/or prophylactic effects against various diseases related to the underexpression of such human haemopoietic maturation factor polypeptide.		

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HAEMOPOIETIC MATURATION FACTOR

This invention relates to newly identified polynucleotide sequences, polypeptides encoded by such sequences, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptide of the present invention is a haemopoietic factor.

Various growth factors have been discovered, studied and utilized (cellular and molecular biology, edited by the Japanese Tissue Culture Association, Asakura Shoten (1987)). Such cell growth factors include epidermal growth factor, platelet derived growth factor, acidic fibroblast growth factor and basic fibroblast growth factor. All these factors have been isolated based upon growth promotion of fibroblast cells. However, these factors have also been found to display widely ranging activity and poor specificity.

Accordingly, recent attempts have been made to search for growth factors specifically acting on functionally differentiated cells. As a result, growth factors such as keratinocyte growth factor and hepatocyte growth factor have been isolated, thus creating the possibility that these factors could be used to treat diseases vulnerable to

their specific action spectra. Another growth factor which has been isolated is disclosed in European Patent Application No. 92102385.9 applied for by Takeda Chemical Industries, Ltd. disclosing a glia activating factor which has glial cell growth promoting activity, and the DNA encoding for that polypeptide.

Haematopoiesis is the production of blood cells. The major haematopoietic tissues are bone marrow, spleen, lymph nodes and thymus. Haematopoiesis in the human embryo begins in the second week of life. Bone marrow appears in the embryo in the second month, and it becomes the dominant haematopoietic organ in the latter half of gestation and throughout postnatal life. The bone marrow contains stem cells that give rise to all cells of the haemopoietic series. All of the blood cells except T-lymphocytes are produced in the marrow. Haematopoietic stems cells differentiate into the mature blood cells in response to the action of haemopoietic maturation factor. Accordingly, a haematopoietic growth factor which could be purified and isolated, would have extensive therapeutic value in the treatment and diagnosis of blood cell and other diseases.

In accordance with one aspect of the present invention, there is provided a novel polypeptide which is a haemopoietic maturation factor, as well as analogs and derivatives thereof. The haemopoietic maturation factor of the present invention is of human origin.

In accordance with another aspect of the present invention, there is provided a polynucleotide (DNA or RNA) which encodes such polypeptide.

In accordance with still another aspect of the present invention, there is provided a procedure for producing such polypeptide by recombinant techniques.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptide, or DNA sequence encoding such polypeptide for

therapeutic purposes, for example stimulating differentiation and proliferation of cells of haemopoietic or neural origin.

In accordance with yet another aspect of the present invention, there is provided an antibody against the haemopoietic maturation factor.

In accordance with yet another aspect of the present invention, there is provided a composition which is employed as an antagonist to haemopoietic maturation factor, e.g., an antibody against such polypeptide which may be used to inhibit the action of such polypeptide, for example, in the treatment of T-cell deficient related disorders.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are meant only as illustrations of specific embodiments of the present invention and are not meant as limitations in any manner.

FIG. 1 shows the polynucleotide sequence which encodes for the haemopoietic maturation factor polypeptide. It also shows the amino acid sequence of the haemopoietic maturation factor polypeptide. The standard three letter abbreviation has been used to depict the amino acid sequence.

FIG. 2 shows the homology of the haemopoietic maturation factor to glia maturation factor β .

FIG. 3 illustrates the banding characteristics of the purified haemopoietic maturation factor protein after bacterial expression, and purification.

FIG. 4 is a Northern Blot analysis indicating the organs in the human body in which the haemopoietic maturation factor is predominantly found.

FIG. 5 illustrates that Hela cell growth is inhibited in the presence of haemopoietic maturation factor.

In accordance with one aspect of the present invention, there is provided a DNA sequence (and corresponding RNA sequence) as set forth in Figure 1 of the drawings and/or DNA (RNA) sequences encoding the same polypeptide as the sequence of Figure 2 of the drawings, as well as fragment portions, derivatives, analogs and all allelic variants of such sequences.

In accordance with another aspect of the present invention, there is provided a polynucleotide which encodes the same polypeptide as the polynucleotide of the cDNA clone deposited as ATCC deposit number 75514, deposited on August 4, 1993, and/or fragments, analogs, derivatives or allelic variants of such polynucleotide.

In the case of DNA, DNA may be single stranded or double stranded, and if single stranded the DNA sequence may be the "sense" strand shown in Figure 1 or the one complementary thereto.

The polynucleotide (DNA or RNA, preferably DNA) includes at least the portion coding for the polypeptide, which coding portion may be the same as that in the deposited clone or may be different than that in the deposited clone provided that it encodes for the same polypeptide or an allelic variant thereof. The coding portion preferably encodes at least the mature form of the protein of the present invention.

The present invention further relates to polynucleotide sequences which hybridize to the hereinabove-described polynucleotide sequences if there is at least 50% and preferably at least 70% identity between the sequences. In another preferred embodiment, the present invention relates to polynucleotide sequences which hybridize under stringent conditions to the hereinabove-described polynucleotide sequences. As herein used, the term "stringent conditions" means hybridization will occur if there is at least 95% and preferably at least 97% identity between the segments. Thus, the present invention includes DNA (RNA) sequences encoding allelic variant forms of the peptide encoded by the DNA of Figure 1. Thus, the present invention provides isolated DNA (RNA) encoding for a naturally occurring human polypeptide which is a haemopoietic maturation factor, as well as allelic variants thereof.

The present invention further relates to a polypeptide which is a haemopoietic maturation factor, and which has the structure shown in Figure 2, as well as allelic variants thereof, and analogs, fragments and derivatives thereof which have the same function as the naturally occurring polypeptide.

The polypeptide of the present invention is mainly expressed in haemopoietic tissues and , as such, represents an haemopoietic maturation factor for controlling cells of haemopoietic origin and cells with which they interact.

The present invention further relates to a polypeptide encoded by the DNA contained in the clone deposited as ATCC number 75514 on August 4, 1993 as well as analogs, fragments, derivatives and allelic variants thereof. These deposits will be maintained under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the purposes of Patent Procedure. These deposits are provided merely as a convenience and are not an admission that a deposit is required under 35 U.S.C. § 112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with the description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The polypeptide of the present invention is preferably provided in an isolated form, and preferably is purified.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or DNA or polypeptide, separated from some or all of the coexisting materials in the natural system,

is isolated. Such polynucleotide could be part of a vector and/or such polynucleotide or polypeptide could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

In a preferred embodiment, the haemopoietic maturation factor is a full length mature human haemopoietic maturation factor or an allelic or glycosylated variant thereof. The polynucleotide may also encode a preprotein which is processed and secreted from mammalian cells as the mature protein.

The polynucleotide sequence of the present invention may encode for a mature form of the polypeptide or it may also encode for a leader sequence which facilitates secretion of the polypeptide. For example, the desired DNA sequence may be fused in the same reading frame to a DNA sequence which aids in secretion of the polypeptide, for example a leader sequence which functions as a secretory sequence for controlling transport of the polypeptide from the cell of the host. The protein having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the mature form of the protein. The polynucleotide of the present invention may also be fused in frame to a marker sequence, for example, a histidine tag, which allows for purification of the polypeptide.

Thus, the polypeptide(s) of the present invention may be the mature form of the haemopoietic maturation factor of the present invention; or may be in the form of a preprotein or prepolyptide wherein the mature polypeptide includes a leader or secretory sequence; or may be in the form of a fusion protein wherein additional amino acids which aid in, for example, purification of the polypeptide are fused to the mature or preprotein at either the 3' or 5' end thereof.

In a preferred embodiment of the present invention, the marker sequence is a hexa-histidine tag added to the coding frame by a bacterial expression vector.

The polypeptide of the present invention is found predominantly in the spleen and thymus and is most concentrated in the leukocytes.

As hereinabove indicated, the present invention also includes variants of the polypeptide which is encoded by the DNA of Figure 1 and/or variants of the DNA contained in the deposited clone, which retains the qualitative activity of such a polypeptide which is a haemopoietic maturation factor. The variant may be a substitutional variant, or an insertion variant or a deletional variant. Such variants can be naturally occurring allelic variants such as for example, those with different glycosylation patterns or substitution at the amino acid level or deletion at the amino acid level.

Such variants may also be produced by site specific mutagenesis. The substitutional variant may be a substituted conserved amino acid or a substituted non-conserved amino acid, and preferably a conserved amino acid.

A polynucleotide encoding a polypeptide of the present invention may be obtained from a cDNA library derived from human early stage kidney tissue, spleen, thymus, or from leukocytes. It contains an open reading frame encoding a mature protein of 142 amino acids in length. The amino acid of the haemopoietic maturation factor has sequence homology to a known growth factor isolated from neural cells. The polypeptide encoded by the polynucleotide is structurally related to human glia maturation factor with 82% identical amino acids and 92% similarity over the entire region. It is also structurally related to bovine glia maturation factor with 82% identical amino acids and

92% similarity over the entire coding region. The polypeptide may be found on the surface of T-cells.

Glia maturation factor is a 17 kd protein found in the brain of mammals with no apparent sequence homology to other known proteins. It is most abundant in the embryo but is also found in later stages of development. The human glial maturation factor controls the differentiation and proliferation of certain cells of neural origin.

Host cells are transformed with the expression vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants or amplifying the haemopoietic maturation factor gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

"Transformation" means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integration. Unless indicated otherwise, the method used herein for transformation of the host cells is the method of Graham, F. and van der Eb, A., Virology 52:456-457 (1973). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used. If prokaryotic cells or cells which contain substantial cell wall constructions are used, the preferred method of transfections is calcium treatment using calcium chloride as described by Cohen, F.N. et al., Proc. Natl. Acad. Sci. (USA), 69:2110 (1972).

"Transfection" refers to the introduction of DNA into a host cell whether or not any coding sequences are ultimately expressed. Cells do not naturally take up DNA. Thus, a variety of technical "tricks" are utilized to bypass natural barriers to gene transfer. Numerous methods of transfection are known to the ordinarily skilled

artisan, for example, CaPO₄ and electroporation. Transformation of the host cell is the indicia of successful transfection.

The polynucleotide of the present invention may be employed for producing a polypeptide by recombinant techniques. Thus, for example, the polynucleotide sequence may be included in any one of a variety of vectors or plasmids for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA's; yeast plasmids; vectors derived from combinations of plasmids and phage DNAs, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other plasmid or vector may be used as long as it is replicable and viable in the host.

As hereinabove described, the appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli, lac or trp, the phage lambda PL promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain a gene to provide a phenotypic trait for selection of

transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Salmonella typhimurium; fungal cells, such as yeast; animal cells such as CHO or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter and an enhancer, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example.

Bacterial: pQE-9 (Qiagen), pBs, phagescript, PsiX174, pBluescript SK, pBsKS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as it is replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described construct. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, 1986).

The constructs in host cells can be used in a conventional manner to produce the gene product coded by the recombinant sequence. Alternatively, the encoded polypeptide can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second

Edition, (Cold Spring Harbor, N.Y., 1989), the disclosure of which is hereby incorporated by reference.

Transcription of a DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin (bp 100 to 270), a cytomegalovirus early promoter enhancer, a polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α -factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli,

Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These PBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is derepressed by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Haemopoietic maturation factor is recovered and purified from recombinant cell cultures by methods used heretofore, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography (e.g., using DNA or nucleotides on a solid support), hydroxyapatite chromatography and lectin chromatography. It is preferred to have low concentrations (approximately 0.1-5mM) of calcium ion present during purification (Price, et al., J. Biol. Chem., 244:917 (1969)).

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more salting-out, aqueous ion exchange or size exclusion chromatography steps. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

The polypeptide of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture) of a polynucleotide sequence of the present invention. Depending upon the host employed in a recombinant production procedure, the polypeptides of the

present invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue (at position minus 1).

In addition to naturally occurring allelic forms of the polypeptide, the present invention also embraces analogs and fragments thereof, which also function as a haemopoietic maturation factor. Thus, for example, one or more of the amino acid residues of the polypeptide may be replaced by conserved amino acid residues.

The haemopoietic maturation factor may be used in the treatment of abnormalities of neural or haemopoietic origin, for example cancer, since the haemopoietic maturation factor may block or retard the growth of cancerous cells.

The polypeptide of the present invention may also be used for treating leukemia. Leukemia is clinically featured by suppression of normal blood cell formation resulting from suppression of normal haemopoiesis by factors produced by leukemic cells. This results in anemia, thrombocytopenia and granulocytopenia. Accordingly, administration of a therapeutically effective amount of haemopoietic maturation factor may be used to overcome the suppression of haemopoiesis by leukemic cells. Haemopoietic maturation factor may also be used for treating other blood related disorders, e.g., hemolysis, polycythemia vera, melogibrosis, hemophilia, and splenomegaly.

Haemopoietic maturation factor may be used in the stimulation of differentiation of mature blood cells in situations where a patient has undergone a bone marrow transplant due to a cancer or other situation. Haemopoietic maturation factor may also be used to stimulate bone marrow cells in vitro for research and diagnostic purposes.

The polypeptide of the present invention may also be employed in accordance with the present invention by expression of such polypeptide *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells such as bone marrow cells may be transduced with a polynucleotide (DNA or RNA) encoding for the polypeptide *ex vivo*, the transduced cells are then provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be transduced by procedures known in the art by use of a retroviral particle containing RNA encoding for the polypeptide of the present invention.

Similarly, transduction of cells may be accomplished *in vivo* for expression of the polypeptide *in vivo*, for example, by procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for transduction *in vivo* and expression of the polypeptide *in vivo*.

These and other methods for administering a polypeptide of the present invention by such methods should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for transducing cells may be other than a retroviral particle, for example, an adenovirus, which may be used to transduce cells *in vivo* after combination with a suitable delivery vehicle.

The polypeptide of the present invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the protein, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

An injection of a composition containing the haemopoietic maturation factor may be prepared by conventional methods using, for example, physiological saline or aqueous solutions containing glucose or other auxiliary agents. The pharmaceutical compositions such as tablets and capsules can also be prepared in accordance with conventional methods. The injections, solutions, tablets and capsules as the pharmaceutical compositions are prepared under aseptic conditions.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptide of the present invention may be employed in conjunction with other therapeutic compounds.

When the haemopoietic maturation factor of the present invention is used as a pharmaceutical, it can be given to mammals (such as humans, mice, rats, hamster, dogs, rabbits and cats), in a suitable vehicle.

When the polypeptide of the present invention is used as a pharmaceutical as described above, it is given, for example, to the above-mentioned subjects in therapeutically effective doses of at least about 10 $\mu\text{g/kg}$ body weight and in most cases it would be administered in an amount not in excess of about 8 mg/kg body weight per day and preferably the dosage is from about 10 $\mu\text{g/kg}$ body weight to about 1 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc.

Each of the cDNA sequences identified herein or a portion thereof can be used in numerous ways as

polynucleotide reagents. The sequences can be used as diagnostic probes for the presence of a specific mRNA in a particular cell type. In addition, these sequences can be used as diagnostic probes suitable for use in genetic linkage analysis (polymorphisms).

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of cDNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the cDNA is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include in situ hybridization, prescreening with labeled flow-sorted chromosomes and preselection by

hybridization to construct chromosome specific-cDNA libraries.

Fluorescence in situ hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 500 or 600 bases; however, clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the clone from which the EST was derived, and the longer the better. For example, 2,000 bp is good, 4,000 is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one

of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that cDNA sequence. Ultimately, complete sequencing of genes from several individuals is required to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

The present invention is further directed to inhibiting haemopoietic maturation factor *in vivo* to reduce and/or eliminate its stimulatory effect by use of antisense technology. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide sequence to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the polypeptide of the present invention, is used to design an antisense RNA oligonucleotide of from 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al, Nucl. Acids Res., 6:3073 (1979); Cooney et al, Science, 241:456 (1988); and Dervan et al, Science, 251: 1360 (1991), thereby preventing transcription and the production of haemopoietic maturation factor.

The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of an mRNA molecule into the haemopoietic maturation factor (antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)).

Alternatively, the oligonucleotides described above can be delivered to cells by procedures in the art such that the anti-sense RNA or DNA may be expressed *in vivo* to inhibit production of haemopoietic maturation factor in the manner described above.

Antisense constructs to haemopoietic maturation factor, therefore, may stimulate cell growth which may be used for treating certain disorders, for example, AIDS, since blocking haemopoietic maturation factor may control T-cell maturation. In this way, antisense constructs may also be used to treat anemia.

The protein, its fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal, monoclonal, chimeric, single chain, Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of polyclonal antibodies.

Antibodies generated against the polypeptide corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptide into an animal or by administering the polypeptide to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies binding the whole native polypeptide. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide. Moreover, a panel of such antibodies, specific to a large number of polypeptides, can be used to identify and differentiate such tissue.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature,

256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention.

The antibodies can be used in methods relating to the localization and activity of the protein sequences of the invention, e.g., for imaging these proteins, measuring levels thereof in appropriate physiological samples and the like.

Antibodies specific to the haemopoietic maturation factor may further be used as antagonists to inhibit the proper functioning of the polypeptide. In this manner, the antibodies may be used in therapy, for example, to stimulate cell growth to treat certain disorders, for example, AIDS and anemia.

Further, such antibodies can detect the presence or absence of haemopoietic maturation factor and, therefore, are indicators of disorders of the haemopoietic and neural system. These antibodies may, therefore, be used as a diagnostic for disorders associated therewith. Prenatal diagnosis may also be particularly useful where the associated disorders are congenital.

Alternatively, an antagonist to the polypeptide of the present invention may be employed which binds to the receptors to which the polypeptide of the present invention normally binds. The antagonist is similar to an inactive form of the polypeptide and may be generated in ways similar to the ways in which antibodies specific to haemopoietic maturation factor are generated. In this way

the action of the haemopoietic maturation factor is prevented and the antagonist may be used therapeutically for stimulation of cell growth to treat certain disorders, for example AIDS. The antagonist may also be used diagnostically to detect the presence or absence of haemopoietic maturation factor.

The present invention will now be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples, certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μ g of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μ l of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μ g of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the

manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. et al., Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units of T4 DNA ligase ("ligase") per 0.5 µg of approximately equimolar amounts of the DNA fragments to be ligated.

Example 1

Expression and purification of the haemopoietic maturation factor.

The DNA sequence encoding for haemopoietic maturation factor (ATCC # 75514) is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' end of the DNA sequence to synthesize insertion fragments. The 5' oligonucleotide primer has the sequence
GACTTCATGAAAAAGACAGATATCGCAATTGCAGTGGCAC-
TGGCTGGTTTCGCTACCGTTGCGCAAGCTGCTTCTGACTCCCTGGTGGTGTGC
contains a BspHI restriction enzyme site and the ompA

leader sequence followed by 21 nucleotides of haemopoietic maturation factor coding sequence starting from the codon following the methionine start codon; the 3' sequence GACTAGATCTACGAAAGAAAGACAACCTTTTC contains complementary sequences to BglII site, and the last 21 nucleotides of haemopoietic maturation factor coding sequence. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-60 (Qiagen Inc., 9259 Eton Ave., Chatsworth, CA 91311). The plasmid vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His) and restriction enzyme cloning sites. The pQE-60 vector was digested with NcoI and BglII and the insertion fragments were then ligated into the pQE-60 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture was then used to transform the *E. coli* strain ml5/rep4 (available from Qiagen under the trademark ml5/rep4). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates containing both Amp and Kan. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in either LB media supplemented with both Amp (100 µg/ml) and Kan (25 µg/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells were grown to an optical density of 600 (O.D.⁶⁰⁰) between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalactopyranoside") was then added to a final concentration of 1mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 3-4 hours. Cells were then harvested by centrifugation. The cell pellet was

solubilized in the chaotropic agent 6 Molar Guanidine HCL. After clarification, solubilized haemopoietic maturation factor was purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag.

(Hochuli, E. et al., Genetic Engineering, Principle & Methods, 12:87-98 Plenum Press, New York (1990)).

Haemopoietic maturation factor (95% pure) was eluted from the column in 6 molar guanidine HCL pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCL, 100mM sodium phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein was dialyzed to 50 mmolar sodium phosphate.

Example 2

Expression of RNA encoding the haemopoietic maturation factor in different tissues.

In order to analyze the expression pattern of the haemopoietic maturation factor RNA was isolated from different human tissues. After total RNA isolation poly A containing RNA was isolated by affinity chromatography on a poly T containing affinity resin. Poly A containing RNA (2 ug) was separated by denaturing gel chromatography and the poly A containing RNA transferred to a membrane by blotting. RNA expression pattern was determined by hybridizing the membrane bound poly A RNA to a radioactive probe spanning the haemopoietic maturation factor gene followed by autoradiography. Figure 4.

Example 3

Haemopoietic maturation factor and inhibition of growth of HeLa cells.

HeLa cells were plated at 10^5 (experiment #1) or 10^4 (experiment #2 and #3) per well in 24 well plates. Cells were grown in DMEM ("Dulbecco's Modified Eagle's Medium") medium (1 ml) containing 5% fetal calf serum penicillium 50 units/ml and streptomycin 50 ug/ml. Four wells were plated for each concentration of haemopoietic maturation factor per experiment. Haemopoietic maturation factor was applied one day after plating. After 3 days cells were trypsinized and counted. Figure 5.

The results indicate that haemopoietic maturation factor inhibits the growth of cancer cells, since Hela cells are forms of cancer cells.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- (i) APPLICANT: KIRKNESS, ET AL.
- (ii) TITLE OF INVENTION: Haemopoietic Maturation
Factor
- (iii) NUMBER OF SEQUENCES: 2
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN,
CECCHI, STEWART & OLSTEIN
- (B) STREET: 6 BECKER FARM ROAD
- (C) CITY: ROSELAND
- (D) STATE: NEW JERSEY
- (E) COUNTRY: USA
- (F) ZIP: 07068
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: 3.5 INCH DISKETTE
- (B) COMPUTER: IBM PS/2
- (C) OPERATING SYSTEM: MS-DOS
- (D) SOFTWARE: WORD PERFECT 5.1
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:
- (B) FILING DATE: Concurrently
- (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: 08/187,186
- (B) FILING DATE: 25 JAN 1994
- (viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: FERRARO, GREGORY D.
(B) REGISTRATION NUMBER: 36,134
(C) REFERENCE/DOCKET NUMBER: 325800-143

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 201-994-1700
(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 600 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGACAGCGGA ACTAAGAAAA GAAGAGGCCT GTGGACAGAA CAATCATGTC TGA CTCCCTT
60
GTGGTGTGCG AGGTAGACCC AGAGCTAACA GAAAAGCTGA GGAAATTCCG CTTCCGAAAA
120
GAGACAGACA ATGCAGCCAT CATAATGAAG GTGGACAAAG ACCGGCAGAT GGTGGTGCTG
180
GAGGAAGAAT TTCAGAACAT TTCCCCAGAG GAGCTCAAAA TGGAGTTGCC GGAGAGACAG
240
CCCAGGTTCTG TGGTTTACAG CTACAAGTAC GTGCATGACG ATGGCCGAGT GTCCTACCCT
300
TTGTGTTTCA TCTTCTCCAG CCCTGTGGGC TGCAAGCCGG AACAACAGAT GATGTATGCA
360
GGGAGTAAAA ACAGGCTGGT GCAGACAGCA GAGCTCACA AAGGTGTTCTGA AATCCGCACC
420
ACTGATGACC TCACTGAGGC CTGGCTCCAA GAAAAGTTGT CTTTCTTTCTG TTGATCTCTG
480
GGCTGGGGGAC TGAATTCCTG ATGTCTGAGT CCTCAAGGTG ACTGGGGACT TGAACCCCT
540
AGGACCTGAA CAACCAAGAC TTAAATAAA TTTTAAATG CAAAAAAAAA AAAAAAAAAA
600

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 142 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Asp Ser Leu Val Val Cys Glu Val Asp Pro Glu Leu
Thr
5 10
15
Glu Lys Leu Arg Lys Phe Arg Phe Arg Lys Glu Thr Asp Asn
Ala
20 25
30
Ala Ile Ile Met Lys Val Asp Lys Asp Arg Gln Met Val Val
Leu
35 40
45
Glu Glu Glu Phe Gln Asn Ile Ser Pro Glu Glu Leu Lys Met
Glu
50 55
60
Leu Pro Glu Arg Gln Pro Arg Phe Val Val Tyr Ser Tyr Lys
Tyr
65 70
75
Val His Asp Asp Gly Arg Val Ser Tyr Pro Leu Cys Phe Ile
Phe
80 85
90
Ser Ser Pro Val Gly Cys Lys Pro Glu Gln Gln Met Met Tyr
Ala

95	100
105	
Gly Ser Lys Asn Arg Leu Val Gln Thr Ala Glu Leu Thr Lys	
Val	
110	115
120	
Phe Glu Ile Arg Thr Thr Asp Asp Leu Thr Glu Ala Trp Leu	
Gln	
125	130
135	
Glu Lys Leu Ser Phe Phe Arg	
140	

WHAT IS CLAIMED IS:

1. An isolated DNA sequence comprising DNA encoding at least the same mature polypeptide as the DNA sequence of Figure 1 or any allelic variants thereof.
2. The isolated DNA of claim 1 wherein said DNA comprises the DNA of Figure 1 encoding for at least the mature polypeptide of Figure 1 and allelic variants thereof.
3. An isolated RNA sequence comprising RNA and allelic variants thereof corresponding to a DNA sequence of claim 1.
4. An isolated DNA sequence comprising a DNA sequence encoding at least a mature polypeptide identical to the mature polypeptide encoded by the DNA sequence contained in ATCC Deposit No. 75514 and allelic variants thereof.
5. The isolated DNA of claim 4 wherein said DNA comprises the DNA contained in ATCC Deposit No. 75514 which encodes the mature polypeptide.
6. An isolated RNA sequence comprising RNA corresponding to the DNA sequence of claim 4.
7. An expression vehicle comprising the DNA sequence of claim 1.
8. An expression vehicle comprising the DNA sequence of claim 4.
9. A polypeptide comprising an amino acid sequence encoded by the DNA sequence of claim 1 or an active fragment, derivative or functional analog thereof.
10. A polypeptide comprising an amino acid sequence encoded by the DNA sequence of claim 4 or an active fragment, derivative or functional analog thereof.
11. A polypeptide comprising an amino acid sequence encoded by the DNA sequence of claim 2 or an active fragment, derivative or functional analog thereof.
12. A polypeptide comprising an amino acid sequence encoded by the DNA sequence of claim 5 or an active fragment, derivative or functional analog thereof.

13. A process for producing a polypeptide comprising: expressing a polypeptide by use of the DNA of claim 1.
14. An antibody against the polypeptide of claim 9.
15. An antibody against the polypeptide of claim 10.
16. Cells engineered with DNA of claim 1.
17. A process for producing cells for expressing a polypeptide comprising: genetically engineering cells with the DNA of claim 1.
18. An antagonist against a polypeptide of claim 9.
19. A method for the treatment of a patient having need of a haemopoietic maturation factor comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 9.
20. A method for the treatment of a patient to inhibit a haemopoietic maturation factor comprising: administering to the patient a therapeutically effective amount of the antagonist of claim 18.
21. An isolated DNA sequence hybridizable to the DNA sequence of claim 1.
22. The method of Claim 19 wherein said therapeutically effective amount of said polypeptide is administered to a patient by providing to the patient DNA encoding said polypeptide and expressing said polypeptide *in vivo*.

FIG. 1A

1 AGACAGCGGAACAAAGAAAGAGGCCCTGTGGACAGAACAAATCATGTCTGACTCCCTG
 -----+-----+-----+-----+-----+-----+-----+
 60 MetSerAspSerLeu

61 GTGGTGTGCGAGGTAGACCCAGAGCTAACAGAAAGCTGAGGAAATTCGCTTCCGAAAA
 -----+-----+-----+-----+-----+-----+-----+
 120 ValValCysGluValAspProGluLeuThrGluLysLeuArgLysPheArgPheArgLys

121 GAGACAGACAATGCAGCCATCATAATGAAGGTGGACAAAGACCGCAGATGGTGGTGCTG
 -----+-----+-----+-----+-----+-----+-----+
 180 GluThrAspAsnAlaAlaIleIleMetLysValAspLysAspArgGlnMetValValLeu

181 GAGGAAGAATTCAGAACATTTCCCCAGAGGAGCTCAAAATGGAGTTGCCGGAGAGACAG
 -----+-----+-----+-----+-----+-----+-----+
 240 GluGluGluPheGlnAsnIleSerProGluGluLeuLysMetGluLeuProGluArgGln

241 CCCAGGTTCTGGTTTACAGCTACAAGTACGTGCATGACGATGCCGAGTGTCTACCCCT
 -----+-----+-----+-----+-----+-----+-----+
 300 ProArgPheValValTyrSerTyrLysTyrValHisAspAspGlyArgValSerTyrPro

301 TTGTGTTTCATCTCTCCAGCCCTGTGGGTGCAAGCCGGAACACAGATGATGTATGCA
 -----+-----+-----+-----+-----+-----+-----+
 360 LeuCysPheIlePheSerSerProValGlyCysLysProGluGlnGlnMetMetTyrAla
 MATCH WITH FIG. 1B

1/5

FIG. 1B

420

+

+

+

480

540

009

225

KME

405

TKV

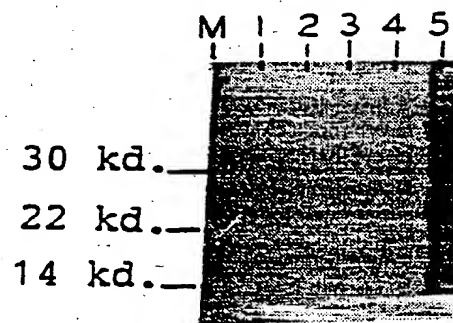
468

11

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FIG. 2

FIG. 3

PURIFICATION OF HAEMOPOIETIC
MATURATION FACTOR PROTEIN

1. 10 ψ PURE PROTEIN
2. 5 ψ PURE PROTEIN
3. 2 ψ PURE PROTEIN
4. 10 ψ PURE PROTEIN (DIF. PREP.)
5. CRUDE PROTEIN

FIG. 4

NORTHERN BLOT
HAEMOPOIETIC MATURATION FACTORM 1 2 3 4 5 6 7 8
| | | | | | | |

2.4 kb —

0.9 kb —

- | | | |
|-------------|--------------------|---------------|
| 1. SPLEEN | 4. TESTES | 7. COLON |
| 2. THYMUS | 5. OVARY | 8. LEUKOCYTES |
| 3. PROSTATE | 6. SMALL INTESTINE | |

FIG. 5

HAEMOPOIETIC MATURATION FACTOR AND HELA CELL GROWTH

Experiment #	Exp.1	Exp.2	Exp.3
Cell Number	(x10 ⁵ ; SD) [% cntl]	(x10 ⁴ ; SD) [% cntl]	(x10 ⁴ ; SD) [% cntl]

HAEMOPOIETIC MATURATION

FACTOR ug/ml

0	3.2; 0.2	6.0; 0.3	5.1; 0.3
0.1	2.9; 0.3 [91]	6.0; 0.3 [100]	4.6; 0.3 [90]
1.0	2.9; 0.3 [91]	4.2; 0.3 [70]	4.4; 0.5 [86]
10.0	1.5; 0.2 [47]	3.7; 0.6 [62]	2.3; 0.2 [45]

SD; STANDARD DEVIATION
[% CNTL]; PERCENT CELLS COMPARED TO UNTREATED oug/ml

HAEMOPOIETIC MATURATION FACTOR

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05186

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C07H 17/00; C12N 15/00; 1/20; C12P 21/06; C07K 13/00; A61K 37/00
 US CL : 536/23.5, 23.51; 435/320.1, 252.3, 69.1; 530/399, 350; 514/12
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5, 23.51; 435/320.1, 252.3, 69.1; 530/399, 350; 514/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS; DIALOG - Biotech Files; GENEMBL sequence databases

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Brain Research, Volume 591, issued 1992, Wang et al, "Polyclonal antibody localizes glia maturation factor β -like immunoreactivity in neurons and glia", pages 1-7, see entire document.	1-13, 16, 17, 19, 21
Y	Journal of Neurochemistry, Volume 57, Number 2, issued 1991, Kaplan et al, "Molecular Cloning and Expression of Biologically Active Human Glia Maturation Factor- β ", pages 483-490, see entire document.	1-13, 16, 17, 19, 21
Y	Journal of Neurochemistry, Volume 60, Number 3, issued 1993, Zaheer et al, "Expresison of Glia Maturation Factor β mRNA and Protein in Rat Organs and Cells", pages 914-920, see entire document.	1-13, 16, 17, 19, 21

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

Special categories of cited documents:	
A document defining the general state of the art which is not considered to be of particular relevance	T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
E earlier document published on or after the international filing date	*X* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	*Z* document member of the same patent family

Date of the actual completion of the international search

09 SEPTEMBER 1994

Date of mailing of the international search report

OCT 13 1994

Name and mailing address of the ISA/US
 Commissioner of Patents and Trademarks
 Box PCT
 Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

ELIZABETH C. KEMMERER

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05186

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-13, 16, 17, 19, and 21

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05186

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings of the National Academy of Sciences USA, Volume 87, issued July 1990, Lim et al, "Complete amino acid sequence of bovine glia maturation factor β ", pages 5233-5237, see entire document.	1-13, 16, 17, 19, 21
Y	Proceedings of the National Academy of Sciences USA, Volume 86, issued May 1989, Lim et al, "Purification and characterization of glia maturation factor β : A growth regulator for neurons and glia", pages 3901-3905, see entire document.	1-13, 16, 17, 19, 21

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/05186

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

I. Claims 1-13, 16, 17, 19, and 21, drawn to nucleic acids, vectors, polypeptides, a process of making polypeptides, transformed cells, and a method of using the polypeptides therapeutically.

II. Claims 14 and 15, drawn to an antibody.

III. Claims 18 and 20, drawn to an antagonist of the polypeptides, and a method of using said antagonist.

IV. Claim 22, drawn to gene therapy.

The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept. For example, the antibodies of Invention II can be used to obtain the nucleic acids of Invention I by expression cloning, and the polypeptides of Invention I can be used to generate the antibodies of Invention II. However, the inventions lack unity in that the nucleic acids, polypeptides, and antibodies are distinct both physically and functionally; the antibodies can be used in different methods, such as various diagnostic and therapeutic methods; and the polypeptides can be used other than to make antibodies, such as therapeutic agents in their own right. Invention III introduces the concept of an antagonist, which is not required by any of the other inventions. Finally, Invention V, drawn to gene therapy, requires consideration of transgenic eukaryotic organisms, efficacious gene expression levels, and containment, which are not required by any of the other inventions.